METHODS FOR AND COMPOSITIONS OF ANTICANCER MEDICAMENTS

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CROSS-REFERENCE TO RELATED PATENT APPLICATIONS

This patent application claims the benefit of U.S. Provisional Patent Application No. 60/463,445 filed April 16, 2003 titled Methods for and Compositions of Anticancer Medicaments.

BACKGROUND OF THE INVENTION

Field of Invention:

The current invention relates to methods for and compositions of anticancer medicaments. Methods include producing nanoparticles and microparticles using antisolvent technology. The invention provides compositions of anticancer medicaments to be used in human or animal treatment of cancerous tissues.

Background:

The formation of fine particles of desired substances in the micro- to nanometer range is an intense area of research. The processes and methods can be extended to a wide variety of materials, including catalysts, chemicals, coatings, explosives, pesticides, polymers and pharmaceuticals. Many supercritical fluid processes have been used to produce fine particles. Most of the research has focused on using either the supercritical fluid as a solvent or an antisolvent. In the Rapid Expansion of Supercritical Solutions (RESS) process, the supercritical fluid is used as the solvent, whereas in Supercritical antisolvent processes (SAS) processes the supercritical fluid is used an antisolvent. The choice of the process depends on the solubility of the material of interest in the supercritical fluid. Some examples of the particles formed using these techniques include steroids (Larson and King, 1985), polystyrene (Dixon et al., 1993), trypsin (Winter et al., 1993) and insulin (Yeo et al., 1993; Winter et al., 1993). Other work has focused on the

formation of fine polymeric particles that contain various drugs for the purpose of controlled drug release (Tom et al., 1992; Mueller and Fischer, 1989). The Debenedetti European Patent Application No. 92119498.1 discloses the formation of protein microparticles using antisolvent precipitation. Schmitt (PCT publication WO 90/03782) discloses the use of antisolvent precipitation for the formation of finely divided solid crystalline powders. Hanna and York (U.S. Patent No. 6,063,138) also disclose a method and apparatus for the formation of particles of given substances using supercritical fluids.

While much research has been performed, SAS can still only be used to produce particles in the 1-10 μm range. Therefore, attempts at adjusting the SAS process have been made in order to address this issue. For example, the use of a coaxial nozzle (PCT publication WO 95/01221) was employed to co-introduce the supercritical fluid and solution, allowing for better atomization of the solution jet. Randolph et al disclose in U.S. Patent Nos. 5,833,891 and 5,874,029 use of an ultrasonic nozzle. Gupta et al expanded the technique in US Patent No. 6,620,351 by employing a vibrating surface in order to atomize the jet into microdroplets and provide a narrow size distribution.

SUMMARY OF THE INVENTION

The present invention provides a method for manufacturing very small particles of anticancer molecules and poorly water soluble molecules comprising the following: providing a contained space, applying a solution having at least a solvent and the anticancer molecules on or close to a surface vibrating at a desired frequency within the contained space, applying a compressed antisolvent to the contained space, and choosing the antisolvent such that it is reasonably miscible with the solvent and that it does not dissolve the molecule substantially. The compressed antisolvent is near or above its critical point and in the liquid state. The size of the particles can be changed by changing the amplitude or frequency of vibration. The frequency can be varied from 10 Hz to 1 Ghz but is preferably in the range of 0.5 kHz and 0.5 GHz. The pressure and temperature of the contained space can be controlled and the temperature can be varied between $0.1T_c$ and $5T_c$. The application of the solution and antisolvent is continuous as well as the collection of the particles. The solvent and antisolvent are both selected from the group consisting of ethanol, methanol, hexane, pentanes, dichloromethane, heptanes, carbon

dioxide, ethane, propane, butane, sulfur hexafluoride, fluoroform, chloroform, isobutane, tetrahydrofuran, 1methyl-2-pyrrolidone, dimethyl sulfoxide, dimethyl formamide, dimethyl acetamide and a combination thereof. However, the preferred antisolvent is carbon dioxide.

The present invention also provides for a pharmaceutical composition comprising particles manufactured according to the aforementioned method and at least one stabilizer. The present invention also provides for an intravenous administration composition comprising particles manufactured according to the aforementioned method and at least one stabilizer. The composition further comprises at least one isotonic liquid carrier. This carrier is either saline or dextran. Stabilizers are selected from the group consisting of polysorbate-80, pluronic block copolymers, lecithin, polyethylene glycol, dextran and a combination thereof. The particles are collected inside the contained space in a liquid medium where the liquid medium is aqueous, organic and substantially nonsolvent for the anticancer molecules, or organic with small dissolving power for the anticancer molecules. The liquid medium may also be an isotonic carrier and contain one or more stabilizers. The contained space can withstand pressures close to 50,000 psi and temperatures close to 400°C. The produced solid particles are associated with a desired free energy. The produced particles may be amorphous or crystalline. Different crystal structures can result from the following factors: change in temperature, change of solvent, change of composition of solvents, change of antisolvent, change of antisolvent, change of composition of solvents, adding a mixing means, changing the extend of mixing and a combination thereof. The vibration of the surface is accomplished by a piezo-electric or magneto-restrictive means. The particles manufactured by the aforementioned method can have a particle size range from 0.01nm to 50 microns and 0.01nm to 0.5 microns. The anticancer molecule is also poorly water soluble.

DETAILED DESCRIPTION

Definitions:

T_c refers to

Critical temperature of the substance which is used as the antisolvent. Depending on the context, it can be the critical temperature of the mixture of solvents and antisolvents also. Irrespective of the unit in which it is represented, the embodiments of the present invention

P_c refers to
Critical pressure of the substance which is used as the antisolvent. Depending on the context, it can be the critical pressure of the mixture of solvents and antisolvents also.

Desired free energy refers to
The desired free energy associated with any solid form. For example, amorphous solids have the highest free energy and most stable solid has the least free energy. Possible polymorphs, stable or otherwise may have free energies in the middle.

Anticancer molecule refers to
Any molecule that might have perceived or verified anticancer or antitumor activity.

Water insoluble molecule refers to
Any molecule that has poor water solubility

Description:

The present invention provides a method of designing and manufacturing poorly water soluble molecules. Such molecules could be from a wide variety of fields including, but not limited to, polymers, chemicals, pesticides, explosives, coatings, catalysts and pharmaceuticals. Furthermore, the present invention discloses a method of manufacturing very small particles of anticancer molecules.

A water insoluble molecule, including anticancer molecules or otherwise, is placed in solution. The solution is then loaded into either a pump or pump feeder. A contained space or particle precipitation vessel is pressurized with compressed antisolvent at the

desired pressure and temperature. The compressed antisolvent to be used in the process includes, but is not limited to, ethanol, methanol, hexane, pentanes, dichloromethane, heptanes, carbon dioxide, ethane, propane, butane, sulfur hexafluoride, fluoroform, chloroform, hydrofluorocarbons, chlorofluorocarbons, isobutane, tetrahydrifuran, 1-methyl-2-pyrrolidone, dimethyl sulfoxide, dimethyl formamide, dimethyl acetamide, or a combination thereof. However, the preferred compressed antisolvent is carbon dioxide. The solvent to be used in the process includes, but is not limited to ethanol, methanol, hexane, pentanes, dichloromethane, heptanes, carbon dioxide, ethane, propane, butane, sulfur hexafluoride, fluoroform, chloroform, isobutane, tetrahydrifuran, 1methyl-2-pyrrolidone, dimethyl sulfoxide, dimethyl formamide, dimethyl acetamide.

Vibration of the surface is started by an external control mechanism and the temperature of the vessel is controlled by a water jacket, chiller, heater or other means. Frequency of vibration may be varied from 10 Hz to 1Ghz. Varying either, or both, frequency and amplitude of vibration can change particle size. Pressure of the system is controlled by a back pressure regulator. A filtering element is provided to retain the produced particles in the vessel or in subsequent collection vessels to which the particles can be transported. Such transportation can be accomplished by the flow of antisolvent or by any other means.

After reaching the desired pressure, temperature and vibration level, all of which are controlled, a solution restriction is opened so that it can be applied on to or close to the vibrating surface. The vibration surface atomizes the droplets or ejects the droplets from the instantaneous film developed on the surface ultimately producing very fine droplets. The film thickness can be as small as a few nanometers to as high as a 20 centimeters. These droplets undergo antisolvent effect when exposed to the antisolvent and start precipitating or crystallizing as very small particles. The antisolvent removes the solvent and takes it to another vessel through a back pressure regulator where it can be removed from solvent and both the solvent and antisolvent can be separated, recycled, reused or discarded. The application of solution and antisolvent is continuous.

Particles are collected contained space or particle precipitation vessel. Antisolvent alone can be used to purge for a period of time to remove any solvent ladden antisolvent in the vicinity and to make sure the particles have the least amount of residual solvent.

In another embodiment particles can be collected in a collection zone that is subsequent in the process to the contained space or particle precipitation vessel. In yet another embodiment particles can be collected in both contained space or particle precipitation vessel and subsequent collection zones. Collection in any of the embodiments can be done in batch, semi-continuous or continuous mode.

In another embodiment of the current invention, a fluid can be inside the contained space or particle precipitation vessel and utilized a means of collection. Such fluids can be water based or organic solvent based and such liquids can also be polymer, natural macromolecule or other typical pharmaceutical excipient based. The fluids can be a solvent to the molecules or a nonsolvent to the molecules. Furthermore, the fluids may contain stabilizers, components to make them isotonic and other components that may be needed so that a final composition can be delivered to the body as a medicament.

Fig 1 illustrates an embodiment of the present invention for designing and manufacturing poorly water soluble molecules. **Fig 2** also illustrates an embodiment that may also be utilized for the manufacturing very small particles of anticancer molecules.
A secondary vessel was used to collect the particles at two different places. A third vessel was used to collect the solvent when the CO_2 was depressurized. This is described in **Fig.2**. **Fig. 3** illustrates another embodiment of the present invention where liquid collection can be utilized.

Particles were characterized through several methods. Scanning electron microscope imaging provided the morphology and size information. X-ray diffraction measurements revealed that the produced-particles were highly crystalline in nature. Further characterization using laser diffraction and dynamic light scattering (Photon correlation spectroscopy) provided size distribution information.

The produced particles may be made into a pharmaceutical composition by stabilizing them in an isotonic suspension.

In another embodiment, the fluids may contain stabilizers, components to make them isotonic and other components. The addition of these stabilizers and components in the fluid provides the elements needed for a composition of the particles, stabilizer(s) and component(s) that can be delivered to a human, animal or other organism as a medicament. The final composition could be a solution or a dispersion. The administration of the composition could be done through intravenous, intramuscular, interperitonial, subcutaneous, inhalation or by any other administration means.

In another embodiment, particles from any of the collection methods used in the present invention may be added to stabilizers, components to make them isotonic and other components to provide the elements needed for a composition as a medicament for delivery to a human, animal or other organism. The final composition could be a solution or a dispersion. The administration of the composition could be done intravenously or by any other method utilizing injection.

The following examples clearly illustrate the present invention:

Solutions of paclitaxel in methanol and ethanol are used in the present invention. Carbon dioxide is used as the antisolvent. The following table summarizes the conditions used for paclitaxel nanoparticle formation studies. This table provides a design with pressure and temperature maintained at 75 bar and 35 °C. It was inferred from phase behavior studies that a pressure below 100 bar and temperature around 35 °C would be an optimal condition for maximum yield of particles.

Pressure	Capillary	Temperature	Conc	CO2 Flow	Sol Flow	Solution Injection	Purge Time With antisolvent, CO2	Vibration Amplitude measured in terms of power input
bar	micron	°C	mg/mL	g/min	mL/min	Time,min	min	Watts
75	100	35	30	50	0.5	30	60	0
75	100	35	30	50	0.5	30	60	200
75	100	35	30	50	2	15	60	200
75	100	35	5	50	0.5	120	60	200
75	100	35	5	50	2	45	60	200
75	100	35	5	50	0.5	88	60	0
75	100	35	5	50	2	37	60	0
75	100	35	17.5	50	1.25	30	60	100
75	100	35	17.5	50	1.25	30	60	100
75	100	35	30	50	0.5	20	60	0
75	100	35	30	50	0.5	30	60	200
75	100	35	30	50	2	15	60	0
75	100	35	30	50	2	15	60	200
75	100	35	5	50	2	45	60	200
75	100	35	5	50	0.5	120	60	0
75	100	35	17.5	50	1.25	30	60	100
75	100	35	17.5	50	1.25	30	60	100
200	100	60	15	50	1	30	60	0

Table 1. Experimental conditions explored for the paclitaxel with various solvents (ethanol, methanol) with frequency at 20 kHz and 40 kHz

Exp #	Solution Flow rate	Vibration Amplitude measured in terms of power input	Sol.Conc	CO2 Flow	T,	P	Solution injection time	Purge Time With antisolvent, CO2
	ml/min	W	mg/mL	g/min	C	bar	min	Min
1	2	0	5	50	35	75	30	120
2	0.5	200	5	50	35	75	120	120
3	2	200	5	50	35	75	30	120
4	2	0	5	50	60	75	30	120
5	2	200	5	50	60	75	30	120
6	0.5	0	5	50	35	75	60	120
7	0.5	200	5	50	60	75	120	120
8	0.5	0	5	50	60	75	60	120
9	1.25	100	5	50	47.5	75	45	120
10	1.25	100	5	50	47.5	75	45	120

Table 2. Experimental conditions explored for the camptothecin in various solvents (dimethyl sulfoxide, dimethyl formamide)

Scanning electron microscope pictures in Figure 4 provide information about particle size and morphology information. The captions at the bottom of each micrograph list the conditions and can also be interpreted using the table above.

In addition to the particle size distribution measurements, x-ray diffraction patterns of the produced powder were measured. A portion of each sample was back-loaded into an XRD holder for analysis. The samples were run on a Philips XRD unit from 4.0 to 34° 2θ at 1.0°/min with a step size of 0.05° using graphite monochromatized copper radiation. The following graph summarizes the XRD patterns of the samples.

Further characterization of the particle size distribution through light scattering techniques provided the following information. Selective results are summarized in Figures 5 through 11 with appropriate sample names.

The following tables show additional experiments that were performed in order to demonstrate the present invention.

Row #	T	P	Sol.Flow	CO_2 Flow	Vibration	Sol.Conc	Sol.Flow time	Antisolvent (CO_2) purge time
	C	bar	ml/min	g/min	watts	mg/ml	min	min
1	35	75	0.5	100	0	20	40	60
2	35	75	0.5	100	200	20	40	60
3	35	75	2	100	0	20	10	60
4	35	75	2	100	200	20	10	60
5	70	75	0.5	100	0	20	40	60
6	70	75	0.5	100	200	20	40	60
7	70	75	2	100	0	20	10	60
8	70	75	2	100	200	20	10	60
9	52.5	75	1.25	100	100	20	16	60
10	35	75	1.25	100	100	20	16	60
11	70	75	1.25	100	100	20	16	60
12	52.5	75	0.5	100	100	20	40	60
13	52.5	75	2	100	100	20	10	60
14	52.5	75	1.25	100	0	20	16	60
15	52.5	75	1.25	100	200	20	16	60

Table 3. Experimental conditions used in producing the particles as per the current invention using dichloromethane as solvent

P	Capillary	T Or T range	Conc	CO2 Flow	Sol Flow	Solvent	Solution injection time/Antisolvent Purge Time	Vibration amplitude Measured as power input
bar	micron	C	mg/mL	g/min	mL/min		min	Watts
100	40	35	40	50	0.5	Methylene Chloride	15/60	0
75	100	35	40	100	2	Methylene Chloride	15/60	100
75	100	52.5	40	100	1.25	Methylene Chloride	22190	100
75	100	35	20	100	0.5	Methylene Chloride	40/60	0
75	100	35	20	100	0.5	Methylene Chloride	40/60	200
75	100	35	20	100	2	Methylene Chloride	10/60	0
75	100	35	20	100	2	Methylene Chloride	10/60	200
75	100	70	20	100	0.5	Methylene Chloride	40/60	0
75	100	70	20	100	2	Methylene Chloride	10/60	0
75	100	70	20	100	2	Methylene Chloride	10/60	200
75	100	46/58	20	100	1.25	Methylene Chloride	16/60	100
75	100	30/36	20	100	1.25	Methylene Chloride	16/60	100
75	100	44/55	20	100	0.5	Methylene Chloride	40/60	100
75	100	44/56	20	100	2	Methylene Chloride	10/60	100

Table 4. Experimental conditions used in producing the particles as per the current invention using dichloromethane as solvent